RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 1 |334-342| January - March | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

A SIMPLE SENSITIVE AND RAPID GAS CHROMATOGRAPHY METHOD FOR DETECTION AND QUANTIZATION OF METFORMIN IN METFORMIN TABLET FORMULATION, BY DIRECT INJECTION, USING MASS DETECTOR

M. D. D'Souza[1,2,✉], P. Dwivedi[1], R. S. Lokhande[1], T. Anvekar[2], S. K. Sharma[2] and A. J. D'Souza[2]

[1]School of Basic Sciences, Jaipur National University, Jaipur-302017, (Rajasthan), India
[2]Department of Chemistry, St. Xavier`s College, Mapusa, Bardez Goa, 403 507 India
✉Corresponding Author: melwindsouza3890@gmail.com

ABSTRACT

Metformin (1, 1- dimethyl biguanide) represents the "gold standard" for the treatment of diabetes mellitus type 2. Despite its important role in reducing mortality and morbidity in the diabetic population, metformin is associated with an increased risk of stroke. A simple, novel, specific, and sensitive gas chromatography-mass spectrometry method (GC-MS) for the determination of metformin was developed. Several parameters such as reaction time and temperature were optimized. There was no interfering peak from the blank at the retention time of metformin. Linearity was demonstrated over the concentration range of 4.024–24.144ppm with a coefficient of determination (R2) above 0.9999. Accuracy and precision were within acceptable limits. The method was fully validated using parameters such as specificity, linearity, limits of detection and quantification, precision, accuracy, recovery, range, robustness, and ruggedness. The developed method was successfully applied for the identification and quantification of metformin in the market tablet formulation.

Keywords: Metformin, Accuracy, Precision GC-MS/MS, Robustness, Ruggedness.

RASĀYAN*J. Chem.*, Vol. 15, No.1, 2022

INTRODUCTION

N, N-Dimethylimidodicarbonimidic diamide common name 'Metformin', a biguanide derivative, is an orally administered antihyperglycemic drug that has been widely used in the treatment of type-2 diabetes for decades.[1-2] Quantification of metformin in biological matrices is required for pharmacokinetic studies, therapeutic drug monitoring, evaluation of bioequivalence of commercially available pharmaceutical formulations, and optimization of dosing especially in combination therapy. Furthermore, the safety of metformin in patients with renal or hepatic insufficiency or other contraindications should be assessed because of the risk of lactic acidosis. Lactic acidosis is a rare and adverse side effect of metformin. In metformin-associated lactic acidosis, metformin accumulation may occur and its plasma concentration may exceed[3-4] 50 mg/L.

Various analytical methods were developed to determine metformin in biological samples.[5-10] These include liquid chromatography[11-12], tandem mass spectrometry, electrochemistry[13-15], gas chromatography[16-19], and capillary electrophoresis.[20-21] Metformin is a highly polar molecule, thus its analysis by reversed-phase chromatography is inconvenient.[22-23] To achieve a reasonable retention time and efficient chromatographic separation, different modes of chromatographic methods such as reversed-phase ion-pairing; normal phase, HILIC, and cation exchange chromatography were developed for the determination of metformin levels in tablet formulations. Metformin in these chromatographic methods was monitored through ultraviolet[24] and tandem mass spectrometry[25] detection. Several GC methods for the analysis of metformin in API and tablet formulation have been published, including GC coupled to a nitrogen phosphorus detector (NPD) and an electron capture detector (ECD). NPD and ECD allow for selective and sensitive detection for drug analysis. However, using MS as the detector not only provides

http://dx.doi.org/10.31788/RJC.2022.1516642

good sensitivity and specificity but also provides additional information from mass spectral data, which is useful in confirming the identity of the drug. The aim of the present studies is a simple sensitive and rapid GC method for detection and quantization of metformin in metformin tablet formulation by direct injection using a mass detector. After full validation of the proposed method, it was finally applied for the identification and quantification of metformin in market tablet formulation.

EXPERIMENTAL

Chemicals and Reagents

Metformin hydrochloride (1,1-dimethyl biguanide hydrochloride) was supplied by Sigma-Aldrich. Metformin tablets were obtained from CIPLA pharma ltd, India. Methyelendichloride and methanol were of GC grade. Purified water was used throughout the study. Other reagents were of AR grade.

Instrumentation and GC-MS Conditions

GC-MS analysis was performed on a Shimadzu GCMS QP 2020plus equipped with a 5973N mass selective detector. A Restek Rtx-624 with guard-column (30 m x 0.32 mm I.D., 1.8 µm) was used for the separation. A split injection (10 mL) was made with a split ratio of 10:1. Helium was used as the carrier gas at a flow rate of 1.5 mL min. The initial oven temperature was set at 60oC, and then the temperature was increased to 240oC at a rate of 15oC min. The total run time was 30min. The MS detector was operated in electron impact ionization mode, and selected ion monitoring mode was used for the identification and quantification of metformin and internal standard. The mass fragments identified were: m/z 130, 86, and 60 for metformin. The ions m/z 80 for metformin were selected for quantification. The electron multiplier of the MS detector was set at 106eV. The mass spectrometer was switched on after a 3.05 min solvent delay time.

Preparation of Standard Solutions

To prepare a stock solution (100 ppm), metformin was accurately weighed and then dissolved in methanol. Working standard solutions were prepared by serial dilution of the stock solution with methanol, solutions were kept at 4^{o}C.

Preparation of Tablet Formulation Samples Obtained From theMarket

A quantity of 20.50mg metformin from the crushed tablet was weighed and transferred into a 100mL volumetric flask, diluted up to the mark with methanol. An aliquot of 2.0 mL from solution was pipetted into a 20mL volumetric flask; content was diluted up to the volume with diluent.

Method Validation

Specificity

The specificity of the method was assessed by analyzing blank methanol samples. This was done to investigate whether there had been any interference from the endogenous substance. To evaluate the interference of commonly used medications on metformin, certain medications were added to the blank methanol samples, which were then analyzed by GC-MS. The chromatograms obtained were examined for any interference at the retention times of both metformin tablet formulation and metformin standard. High concentrations of metformin may give carryover peaks from previous injections. After the injection of high concentrations of metformin into tablet formulation samples were run and evaluated to account for this phenomenon.

Linearity and Limits of the Method (LOD and LOQ)

The linearity of the method was assessed by making calibration curves over the concentration range of 4.024–24.144ppm. The calibration curves were constructed by plotting the peak area ratios (metformin standard) versus the nominal concentrations of metformin. The coefficient of determination (R2) and the back-calculating concentration was evaluated for each calibration point. The limit of quantitation (LOQ) was determined based on a signal-to-noise ratio (S/N) of 10 for the least intense diagnostic ion. The acceptable precision and accuracy at the LOQ point are 20% and 80–120%, respectively. The limit of

detection (LOD) was estimated as the analyte concentration, which gave a signal-to-noise ratio of 3 for the least intense diagnostic ion.[26]

Stock Solution Stability

The stability of the stock solutions of metformin was evaluated at room temperature for up to 24 hours. The freshly prepared solutions were injected and then kept at room temperature. These samples were analyzed after 12 and 24hours respectively. At each time interval i.e. at 0, 12, and 24 hours the solutions were injected in triplicate.

Precision

The procedure was evaluated on two levels such as injector repeatability and intra-assay day.

Injection Repeatability

Injection repeatability was carried out by injecting a minimum of six injections of the same solution into the system. A quantity of 19.87 mg metformin standard was weighed and transferred into a 100 mL volumetric flask, diluted up to the mark by methanol. An aliquot of 2.0mL from stock solution was pipette into a 20 mL volumetric flask; content was diluted up to the volume with the mobile phase and injected onto the GC-MS system for the analysis.

Intra-assay Precision

Intra-assay precision is the precision obtained when the assay is performed by a different analyst, different laboratory, and different instrument/column, etc. Different reagents were also used. The results obtained by this experiment are used to identify which of the above factors contribute significant variability to the final result. A quantity of 20.18mg metformin standard was weighed and transferred into a 100 mL volumetric flask, diluted up to the volume with methanol. An aliquot of 2.0 mL from stock solution was pipette into a 20 mL volumetric flask; content was diluted up to the volume with diluents and injected onto the GCMS system for analysis.[27]

Accuracy

The accuracy of recovery was determined at three concentration points (16, 20, and 24ppm). Appropriate volumes of working standard solutions of metformin were spiked into 100 mL of blank methanol and the samples were prepared for GC-MS analysis. For control samples, corresponding to 100% recovery, the same amounts of standard solutions were added to 100 mL of blank methanol samples after extraction and were prepared for GC-MS analysis.[28]

Robustness and Ruggedness

The robustness and ruggedness tests for the GC-MS method were simultaneously carried out using the Plackett–Burman experimental design. Five variables were selected, which were significant in the performance of the method and experimental runs were performed. Experimental runs were done in a random order to minimize the effect of unexplained variability on the response. The peak area ratio of metformin was accepted as a response. The results were assessed using the Minitab statistical software.

RESULTS AND DISCUSSION

GC-MS method Metformin should be converted to its volatile and thermally stable derivative to be analyzed by GC-MS. The study involved the detection and quantization of metformin in metformin tablet formulation, by direct injection using a mass detector. The mass spectrum of metformin is characterized by a base peak at *m/z* 130 formed by hydrogen transfer from the methyl group of the acetyl moiety to the ionized nitrogen followed by α cleavage. Subsequent rearrangement followed by the loss of the formaldehyde radical results in the formation of the more stable $C_3H_8N_3^+$ cation at *m/z* 86. Ionization of the amide oxygen followed by α cleavage gave the CH_3NCH_3 cation at *m/z* 60. The structure of the metformin fragmentation ions is shown in the Fig.-1. The mass spectrum in Fig.-1 shows the analogous peaks at 130, 86, and 60 respectively.

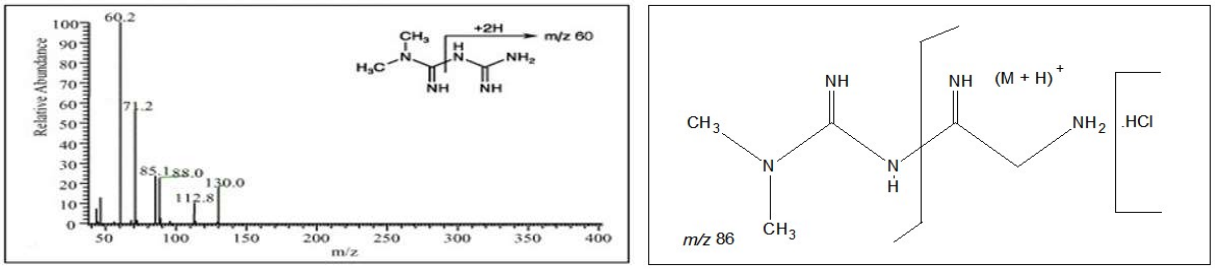

Fig.-1: Mass Spectrum of Metformin Standard and Fragmentation Pattern of Metformin

Method Validation

Specificity and Carryover

Specificity concerning metformin components was determined by analyzing methanol blank samples under controlled conditions. No interference was observed at the retention times of the metformin standard. The samples were analyzed using the proposed extraction procedure and chromatographic conditions to compare them with a methanolic solution of the analyte at a concentration near the limit of quantification. Representative chromatograms of blank and the metformin standard (20ppm) are shown in Fig.-2. No interference was observed at the retention time of the analyte at 5.40 min due to endogenous substances in metformin standard.

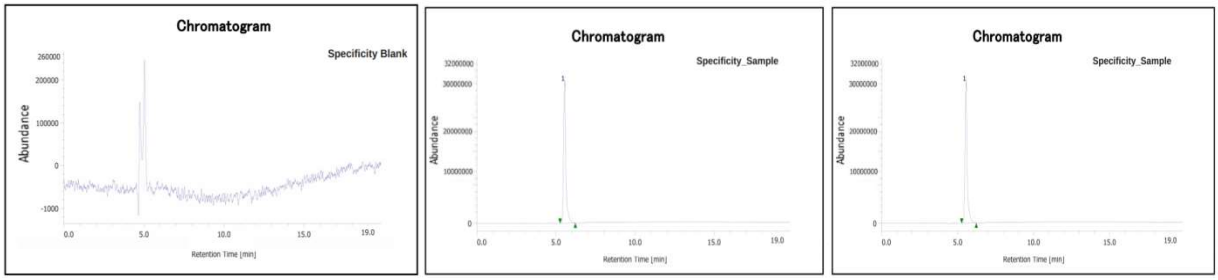

Fig.-2: GC-MS Chromatogram of Blank, Metformin Standard, and Sample

Linearity

The calibration curves showed good linearity within the range 4.024–24.144ppm. The representative linear equation of the calibration curve for the analyte was y = (1,564,461)x - (65,918) with a correlation coefficient of 0.9999, where y is the peak area ratio of the analyte to the IS and the x is the concentration of the analyte. The method was linear over the concentration range of 4.024–24.144ppm with a coefficient of determination (R2) greater than 0.9999.

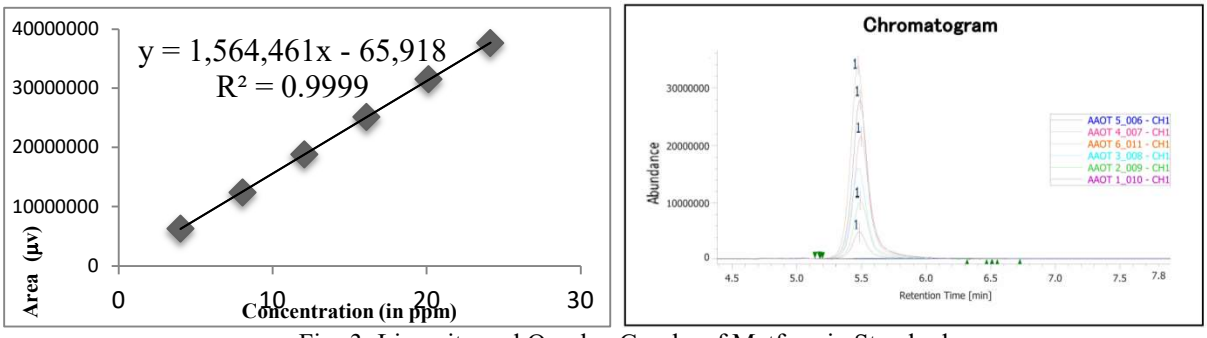

Fig.-3: Linearity and Overlay Graphs of Metformin Standard

Stock Solution Stability

The stability of the stock solutions of metformin was evaluated at room temperature for up to 24 hours. The freshly prepared solutions were injected and then kept at room temperature. These samples were analyzed after 12 and 24 hours respectively. At each time interval i.e. at 0, 12, and 24 hours the solutions were injected in triplicate. The results of the stability experiment are given in Table-2.

Table-1: Linearity Data of MetforminStandard

Linearity Solution Level	Conc. (ppm)	Mean Area
L1	4.024	6304961
L3	12.072	18812340.5
L2	8.048	12407061.5
L4	16.096	25119037
L3	12.072	18812340.5
L6	24.144	37642538.5

Table-2: Standard Solution Stability at 0, 12 and 24 hrs

Inj. No.	Interval	Area (µv)	Mean Area (µv)	Mean % Content	Rel Diff (%)
1	0 hr	31525469	31516690.33	99.89	NA
2		31512457			
3		31512145			
1	12 hrs	31511111	31505466	99.86	0.03
2		31502487			
3		31502800			
1	24 hrs	31498314	31493652.67	99.82	0.07
2		31492457			
3		31490187			

From the results tabulated in Table-2**,** it can be seen that the percentage difference is not more than 2.0% indicating that the results obtained after the specified storage temperatures are comparable with the initial results. It can be concluded that the solutions are stable for 24 h at room temperature.

Precision

Injection Repeatability

The percentage relative standard deviation for all the above parameters is below 2.0. The low values of standard deviation and coefficient of variation indicate good repeatability. It also indicates the satisfactory performance of the system and system suitability for the method.

Table-3: Injection Precision Injection Repeatability for Metformin Tablet Formulations

Sample No.	Conc in ppm	Area (mv)	% Content
Sample-1	20.13	32023876	99.89
Sample-2	20.14	32052254	99.93
Sample-3	20.18	32094567	99.86
Sample-4	20.15	32145620	100.17
Sample-5	20.17	32025478	99.69
Sample-6	20.04	32021148	100.33
Average	NA	NA	99.98
STDEV	NA	NA	0.23
% RSD	NA	NA	0.23

Intra-Assay Precision

The mean, standard deviation, and percentage relative standard deviation were calculated. The peak area was comparable to those of the intra-assay results. The results are in Table-3 and 4. From the precision results, it can be concluded that the method is repeatable, while the intermediate precision results show that the method is reproducible by different analysts on a different day also.

Table-3: Intra-assay Precision of Metformin Tablet Formulations

Sample No.	Conc. in ppm	Area (mv)	% Content
Sample-1	20.11	31022458	100.23
Sample-2	20.19	31054780	99.94
Sample-3	20.09	30698545	99.29
Sample-4	20.13	31326110	101.11
Sample-5	20.04	31015332	100.56
Sample-6	19.82	30687954	100.60

Average	NA	NA	100.29
STDEV	NA	NA	0.63
% RSD	NA	NA	0.63

Table-4: Comparison between Analyst-1 and 2

	Mean % Content	Absolute Difference
Analyst 1	99.98	
Analyst 2	100.29	0.31

Accuracy or Recovery

The percent recovery of the added standard was found to be between 99.92 –100.56 % and with an overall % mean recovery of 100.26. This indicates that the method is free from either positive or negative interferences from the blank. From the above result, it was found that the recovery data of the analyte is within the limit and hence the proposed method is accurate. The result of accuracy (% recovery) of the metformin tablet formulation is tabulated in Table-5.

Table-5: Accuracy Data of MetforminTablet Formulation

Level (%) / pptn	SmplWt (in mg)	Conc (in ppm)	Area (μv)	% Recovery	% Mean Recovery
80_1	16.45	16.45	25519100	100.02	
80_2	16.33	16.33	25515461	100.74	100.31
80_3	16.50	16.50	25634578	100.17	
100_1	20.13	20.13	31435690	100.68	
100_2	20.14	20.14	31356978	100.38	100.56
100_3	20.15	20.15	31445680	100.62	
120_1	24.12	24.12	37299561	99.70	
120_2	24.17	24.17	37399980	99.76	99.92
120_3	24.36	24.36	37899874	100.31	
Overall % Recovery				100.26	
Overall STDEV				0.38	
Overall % RSD				0.38	

Range

The range of an analytical procedure is the interval between the lower and upper concentration (amounts) of the analyte in the sample, for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range for the metformin is evaluated from 20% i.e. 4.024ppm to 120% i.e. 24.144 ppm.

Table-6: Range for Metformin Tablet Formulations

Solution	20% (4 ppm)	120% (24 ppm)
1	6311540	37299561
2	6310169	37399980
3	6315468	37899874
4	6316320	38133451
5	6318284	38433607
6	6320248	38733764
Average	6315338.167	37983372.83
STDEV	3870.70032	566257.7223
% RSD	0.06	1.49

Forced Degradation Studies

The ANOVA test with the Plackett–Burman experimental design was used to identify the effects of each factor on the response (peak area ratio of metformin to IS). The results showed that the effects of selected variables on the response were not significant ($P > 0.05$). In addition, the normal probability plot of the standardized effects was constructed. The normal probabilities (z score) of the rank-ordered variables were plotted on the y-axis, and the standardized variables were plotted on the x-axis. All the points showing the parameters formed a straight line in the probability curves. In other words, the variables for

this study did not have significant effects on the peak area ratios of metformin to IS. Therefore, the method can be considered robust and rugged about the variations tested in this study.

Table-7: Forced Degradation Calculation of Metformin Technical

Condition	% Content					
	Smpl. Wt. (in mg)	Conc. (in ppm)	Area (μv)	% Assay	% Total Imp.	Mass Balance
As such	20.18	20.18	301123456	96.21	0.000	NA
0.05M_$FeCl_3$_24 Hrs	19.99	19.99	300041247	96.77	0.138	100.7
1N_NaOH_24 Hrs	20.09	20.09	300014457	96.28	0.618	100.7
1N_HCl_24 Hrs	20.01	20.01	300061100	96.68	0.112	100.6
3% H_2O_2_24 Hrs	20.13	20.13	300153325	96.13	0.826	100.8
1% Na_2S_24 Hrs	20.19	20.19	300879544	96.08	0.826	100.7
Photo @ 1.2 million lux/Hr	20.12	20.12	300049767	96.15	0.657	100.6
Thermal @ 105°C_24 Hrs	20.11	20.11	300358989	96.30	0.000	100.1

Table-8: Force Degradation of Metformin of Impurity Profile

	% Impurity (by Area Normalization)						
RT about -->	Unk @ 7.150	Unk @ 7.183	Unk @ 7.192	Unk @ 10.875	Unk @ 10.98	Unk @ 14.35	Total Imp.
As such	ND	ND	ND	ND	ND	ND	0.000
0.05M_$FeCl_3$_24 Hrs	0.280	ND	0.194	0.194	ND	0.133	0.801
1N_NaOH_24 Hrs	ND	0.142	0.274	0.274	0.12	ND	0.810
1N_HCl_24 Hrs	ND	0.142	ND	ND	0.138	ND	0.280
3% H_2O_2_24 Hrs	0.520	ND	ND	ND	0.155	ND	0.675
1% Na_2S_24 Hrs	ND	ND	0.539	ND	ND	ND	0.539
Photo @ 1.2 million lux/Hr	0.327	ND	ND	0.255	ND	0.207	0.789
Thermal @ 105°C_24 Hrs	ND	ND	0.526	ND	ND	ND	0.526

Analysis of Metformin Tablet Formulation

Assay of paracetamol tablets formulations of metformin of five different manufacturers has been bought from the Indian market such as Sun Pharma**,** Cipla Pharmaceuticals, Torrent Pharma*,* Panacea Biotec, and Glenmark Pharmaceuticals Limited, etc. Methanol used as solvents were provided by Merck, India. A quantity of around 20mg crushed metformin tablets were weighed and transferred into a 100mL volumetric flask, diluted up to the mark with methanol. Aliquots of the tablets solutions (prepared in methanol) were diluted with methanol (for GC measurement) to obtain final concentrations within the previously mentioned ranges and then treated as under the procedures for GC–MS method.

The developed chromatographic methods were applied for the assay of the metformin in their combined pharmaceutical formulation. Table-9 shows the results obtained for the proposed methods as well as the reference derivative spectrophotometric method. The assay results showed good precision and accuracy as indicated by % recovery, SD, and RSD (%) values. No interfering peaks were observed in the GC chromatograms of the tablets. Results obtained by the developed methods were statistically compared with those of the previously published derivative spectrophotometric method using single-factor analysis of variance (ANOVA) test, which is considered a useful statistical tool for comparison of recovery data obtained from the proposed method. The calculated assay values indicate, no impurities were detected in the five different samples from market tablet formulations.

Table-9: Analysis of Metformin Tablet Formulation

Sample No.	Smpl. Wt. (in mg)	Conc. (in ppm)	Area (mv)	% Assay	% Impurities
Sun pharma	20.06	20.06	301123145	99.78	0.000
Cipla Pharmaceuticals	20.09	20.09	300043647	99.97	0.000
Torrent Pharma	20.12	20.12	300011271	99.99	0.000
Panacea Biotec	20.32	20.32	300061333	99.91	0.000
Glenmark Pharmaceuticals Limited	20.17	20.17	300153399	99.90	0.000

CONCLUSION

The method described in this study is the first GC-MS method developed for the identification and quantification of metformin in market tablet formulation. The method was fully validated according to FDA guidelines. Linearity was achieved over the concentration range of 4.024–24.144ppm. The validation data showed that the developed method is specific, sensitive, precise, accurate, robust, and rugged. Finally, the GC-MS method was demonstrated to be applicable for the analysis of plasma samples from diabetic patients. The run time for the chromatographic separation was demonstrated to be applicable for the analysis of metformin tablet formulation. The run time for the chromatographic separation was <5.5 min, which allowed for high-throughput analysis of metformin in clinical laboratories.

REFERENCES

1. R. J. Dowling, P. J. Goodwin and V. Stambolic, *BMC Medicine*, **9(33),** 2(2011), https://doi.org/10.1186/1741-7015-9-33
2. B. Viollet, B. Guigas, N. S. Garcia, J. Leclerc, M. Foretz and F. Andreelli, *Clinical. Science*, **122(6)**, 253(2012), https://doi.org/10.1042/CS20110386
3. A. M. E. Stades, J. T. Heikens, D. W. Erkelens, F. Holleman and J. B. L. Hoekstra, *Journal of Internal Medicine,* **255(2)**, 179(2004), https://doi.org/10.1046/j.1365-2796.2003.01271.x
4. S. Skrzypek, V. Mirceski, W. Ciesielski, A. Sokołowski and R. Zakrzewski, *Journal of Pharmaceutical and Biomedical Analysis*, **45**, 275(2007), https://doi.org/10.1016/j.jpba.2007.07.010
5. J. G. Swales, R. T. Gallagher, M. Denn, and R. M. Peter, *J. Pharm. Biomed. Anal.*, **55**, 544, (2011), https://doi.org/10.1016/j.jpba.2011.02.030
6. J. G. Swales, R. Gallagher, and R. M. Peter, *Journal of Pharmaceutical and Biomedical Analysis*, **53**, 740(2010), https://doi.org/10.1016/j.jpba.2010.04.033
7. L. Chen, Z. Zhou, M. Shen and A. Ma, *Journal of Chromatographic Science*, **49**, 94(2011), https://doi.org/10.1093/chrsci/49.2.94
8. Y. Wang, Y. Tang, J. Gu, J. P. Fawcett, and X. Bai*, Journal of Chromatography B*, **808**, 215(2004), https://doi.org/10.1016/j.jchromb.2004.05.006
9. H. N. Mistri, A. G. Jangid, and P. S. Shrivastav, *Journal of Pharmaceutical and Biomedical Analysis*, **45**, 97(2007), https://doi.org/10.1016/j.jpba.2007.06.003
10. M. Wang and I. R. Miksa, *Journal of Chromatography B*, **856**, 318(2007), https://doi.org/10.1016/j.jchromb.2007.06.037
11. U. Ebru, *Analytical Methods,* **5(18)**, 4723(2013), https://doi.org/10.36037/IJREI.2020
12. N. Koseki, H. Kawashita, M. Niina, Y. Nagae and N. Masuda, *Journal of Pharmaceutical and Biomedical Analysis*, **36**, 1063(2005), https://doi.org/10.1016/j.jpba.2004.09.007
13. B. G. Charles, N. W. Jacobsen and P. J. Ravenscro, *Clinical Chemistry*, **27(3)**, 434(1981), https://doi.org/10.1093/clinchem/27.3.434
14. S. A. Majidano and M. Y. Khuhawa, *Chromatographia*, **75**, 1311(2012), https://doi.org/10.1007/s10337-012-2321-6
15. J. Brohon and M. No¨el, *Journal of Chromatogr*aphy, **146(1)**, 148(1978), https://doi.org/10.1016/S0378-4347(00)81300-6
16. E. D. L. Putra, N. Nazliniwaty, F. R. Harun and N. Nerdy, *Rasayan Journal of Chemistry*, **13(2)**, 968(2020), http://dx.doi.org/10.31788/RJC.2020.1325645
17. M. S. Lennard, C. Casey, G. T. Tucker, and H. F. Woods, *British Journal of Clinical Pharmacology*, **6(2)**, 183(1978), http://dx.doi.org/10.1111/j.1365-2125.1978.tb00852.x
18. S. B. Matin, J. H. Karam and P. H. Forshan, *Analytical Chemistry*, **47(3)**, 545(1975), https://doi.org/10.1039/C3AY40507A
19. E. P. C. Lai and S. Y. Feng, *Journal of Chromatography B*, **843**, 94(2006), https://doi.org/10.1016/j.jchromb.2006.05.030
20. J. Z. Song, H. F. Chen, S. J. Tian, and Z. P. Sun, *Journal of Chromatography B*, **708**, 277(1998), https://doi.org/10.1016/S0378-4347(97)00635-X

21. Islam Sofiqul, Murugan V and Prema Kumari KB, *Rasayan Journal of Chemistry*, **13(3)**, 1438(2020), http://dx.doi.org/10.31788/RJC.2020.1335775
22. S. R. Polagani, N. R. Pilli, R. Gajula and V. Gandu, *Journal of Pharmaceutical Analysis*, **3**, 9(2013), https://doi.org/10.1016/j.jpha.2012.09.002
23. N. Sultana, M. S. Arayne, N. Shafi, F. A. Sissiqui and A. Hussain, *Journal of Chromatographic Science*, **49(10)**, 774(2011), https://doi.org/10.1093/chrsci/49.10.774
24. K. Chandrasekhar and A. Manikandan, *Rasayan Journal of Chemistry*, **14(2)**, 665(2021), http://dx.doi.org/10.31788/RJC.2021.1425740
25. Ari Wibowo, Shabrina Nurbaiti, Vitarani Dwi Ananda Ningrum, *Advanced Material Research*, **1162**, 173(2001), https://doi.org/10.4028/www.scientific.net/AMR.1162.173
26. F. J. Firdaus F. J. Sami, N. H. Soekamto, and J. Latip, *Rasayan Journal of Chemistry*, **14(2)**, 676(2021), http://dx.doi.org/10.31788/RJC.2021.1425976
27. R. Batubara, B. Wirjosentono, A. H. Siregar, U. Harahap and Tamrin, *Rasayan Journal of Chemistry*, **14(2)**, 751(2021), http://dx.doi.org/10.31788/RJC.2021.1426021
28. I. Sopyan, D. Dwiputri and M. Muchtarid, *Rasayan Journal of Chemistry*, **13(4)**, 2207(2020), http://dx.doi.org/10.31788/RJC.2020.1346045

[RJC-6642/2021]